Reference: *Biol. Bull.*, **138**: 262–271. (June, 1970)

MATURATION OF THE GONADS AND SEASONAL CYCLE OF THE PLANKTONIC LARVAE OF THE OPHIUROID *AMPHIURA CHIAJEI* FORBES [1]

LUCIENNE FENAUX

Chargée de recherche au C.N.R.S. Station Zoologique, Villefranche-sur-Mer (06)

Amphiura chiajei is a little Ophiuroid known in the Mediterranean Sea and in the Atlantic Ocean; it is found from the coasts of Norway to Angola, also along the British Isles and Faroe and the Azores. It produces a planktonic larva, the development of which I have studied (Fenaux, 1963). Although this species is abundant in the Mediterranean, there are no previous records of the presence of its larvae. Only Müller (1853) describes an ophiopluteus from the Adriatic Sea, which seems to be related to *A. chiajei.*

In order to analyze the modalities of laying for a given species, it is as much important to know the conditions in which it takes place normally (and namely to precisely determine the periods during which the larvae appear in the plankton) as to follow the changes taking place during their gonad maturation. For this reason, in the course of my research on the ecological aspects of the reproduction of mediterranean Echinoids and Ophiuroids (Fenaux, 1968 b), I have studied the seasonal variations of gonads and the periodic presence of planktonic larvae of *A. chiajei.* These results appear in the present paper.

Materials and Methods

For the study of maturation of their gonads, ophiuroids were collected twice a month in samples of black mud from the inner basin of Villefranche Bay between April 1965 and March 1966. The seasonal cycle of their ophioplutei was observed in the plankton hauls made regularly over a period of three years (April 1961–April 1964).

The number of male and female ophiuroids whose gonads were examined between April 1965 and April 1966 in either histological sections or smears is indicated below.

	A⁶⁵	M	J	A	S	O	N	D	J⁶⁶	F	M
Males	12	34	28	17	27	26	15	18	29	28	9
Females	14	27	16	18	21	18	10	14	20	10	21
Indeterminable	3	3							1		

The average size of the ophiuroids was 7 mm (diameter of the disc, arms not included); they were all dioecious. Gonads were examined either as smears or in 7 microns histological sections after fixation in the fluids of Bouin, Carnoy or

[1] This study is derived from my state doctor's thesis on the ecological aspects of the reproduction of Echinoids and Ophiuroids of Villefranche-sur-Mer, submitted in June 1968.

Flemming. Slides were stained with Alcian blue, Groat's haematoxylin, Cleveland or Metanyl yellow, or treated with the trichrome stain of Gabe and Martoja-Pierson (1957). These techniques have allowed me to follow the development of the gonads, the stages of which have been differentiated according to the classification adopted by Fuji (1960) for the sea-urchins *Strongylocentrotus nudus* and *S. intermedius.*

I have distinguished six stages of development which are identified as follows:

Stage 0. Gonads are very small and sex cannot be determined even after microscopic examination. I found this stage in seven ophiuroids collected in April and May 1965 and January 1966. These were *Amphiura* whose diameter did not exceed 4 mm (indeterminable ophiuroids mentioned above).

Stage 1. This is the stage which follows the shedding of sexual products. The gonads have a brown-red color. In males, spermatogonia line the wall of the follicle (Fig. 1 A). Within the gonad, one observes non-ejected spermatozoa, left over from the preceding maturation. In females, at the beginning of this stage, the oogonia and small primary oocytes are united in a tissue which is well stained by Alcian blue; this is the nutritive phagocytotic tissue. In certain cases, few large oocytes, remaining from the preceding maturation, can be observed inside the gonad (Figs. 1 A and B). At the end of this stage, the oocytes have invaded the ovary; they are small, 15 to 30 microns in diameter.

Stage 2 (Growth stage). In males, the spermatid layer is thick (Fig. 1 B). In females, there exist two types of oocytes: the first type whose average size is 30 microns and the second type, more numerous, whose average size is 60 microns (Fig. 2 C). On sections treated with the trichrome stain, the larger oocytes are paler and have a dark edge. This differentiation is equally evident in the sections stained with Alcian blue and Groat's haematoxylin. Moreover, the cytoplasm of the large oocytes shows the mesh-like appearance that Delavault (1960) described in the large oocytes of the asteroid *Echinaster sepositus.*

Stage 3 (Pre-maturation stage). In males, spermatogenesis is in progress. As has been observed in *E. sepositus* (Delavault, 1960), the growth of germinal tissue towards the center of the gonad appears as small columns (Fig. 1 C). In females, this stage is characterized by a large increase in the size of the oocytes (average diameter 130 microns). The secondary oocytes cannot be seen in the lumen of the acini (Fig. 2 D).

Stage 4 (Maturation stage). In males, the gonads are filled with spermatozoa (Fig. 1 D). In females, I never observed secondary oocytes on the numerous slides or smears examined. The problem of maturation in the females will be dealt with below.

Stage 5 (Shedding stage). In males, the non-ejected spermatozoa form a mass isolated from the wall of the gonad (Fig. 1 E). In females, I have considered as being in stage 5 those gonads which, although they do not show secondary oocytes, display an evolution in comparison with the pre-maturation stage. In fact, I observe in them a growth of primary oocytes; and in the spaces formed, most likely by the expulsion of a certain number of eggs, appears a vesicular tissue. In addition, certain large oocytes have a degenerating cytoplasm (Fig. 2 E).

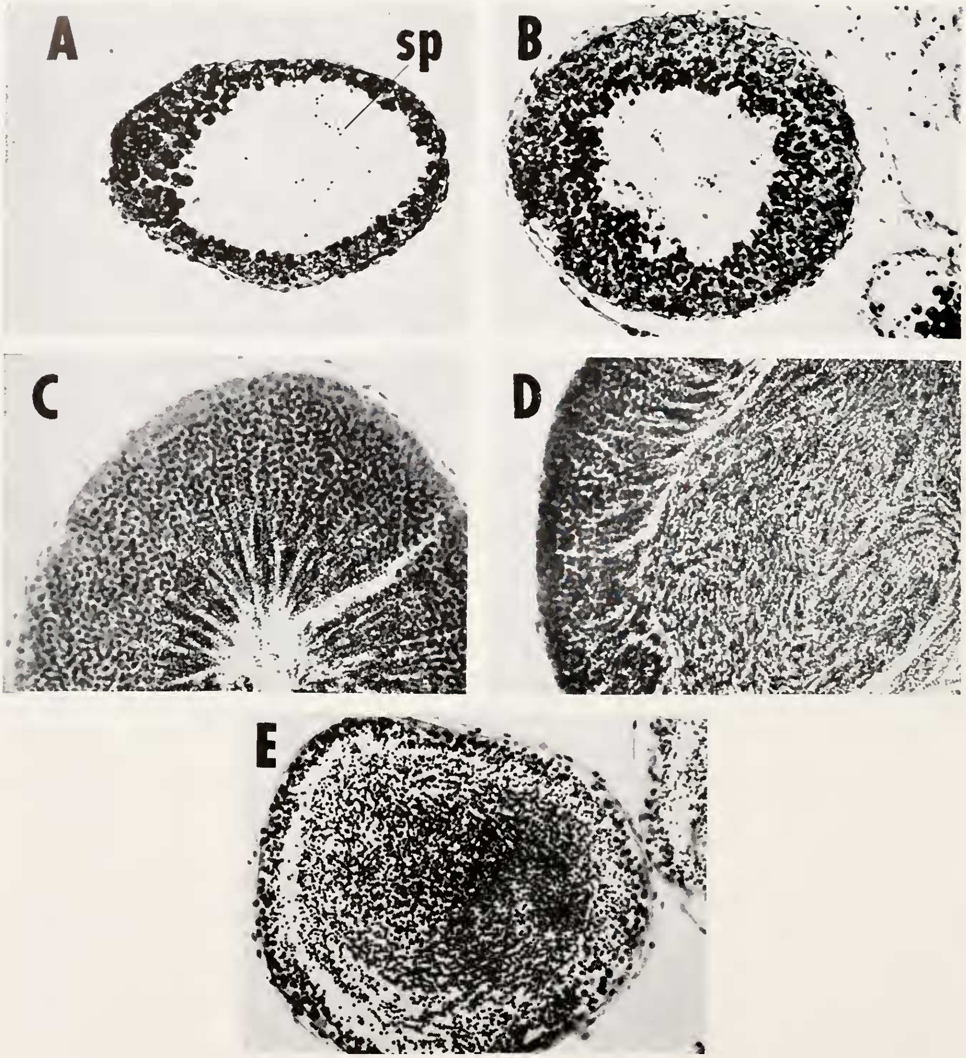

FIGURE 1. Histological sections of gonads of *Amphiura chiajei;* A. Stage 1 testis, in the center of the gonad, few spermatozoa not expelled (sp.), specimen collected in October 1965, × 300; B. Stage 2 testis, February 1966, × 300; C. Stage 3 testis, May 1965, × 120; D. Stage 4 testis, August 1965, × 120; E. Stage 5 testis, October 1965, × 120.

SEASONAL EVOLUTION OF THE GONADS

A. In males

Spring 1965. In April, of the 12 ophiuroids examined, 1 is at stage 1, 5 have gonads at stage 2 and in the remaining, spermatogenesis is in progress (Fig. 3 A). In May, nearly all the ophiuroids have arrived at the stage of maturity. As early as the month of June the stage 5 appears: it is not yet numerous.

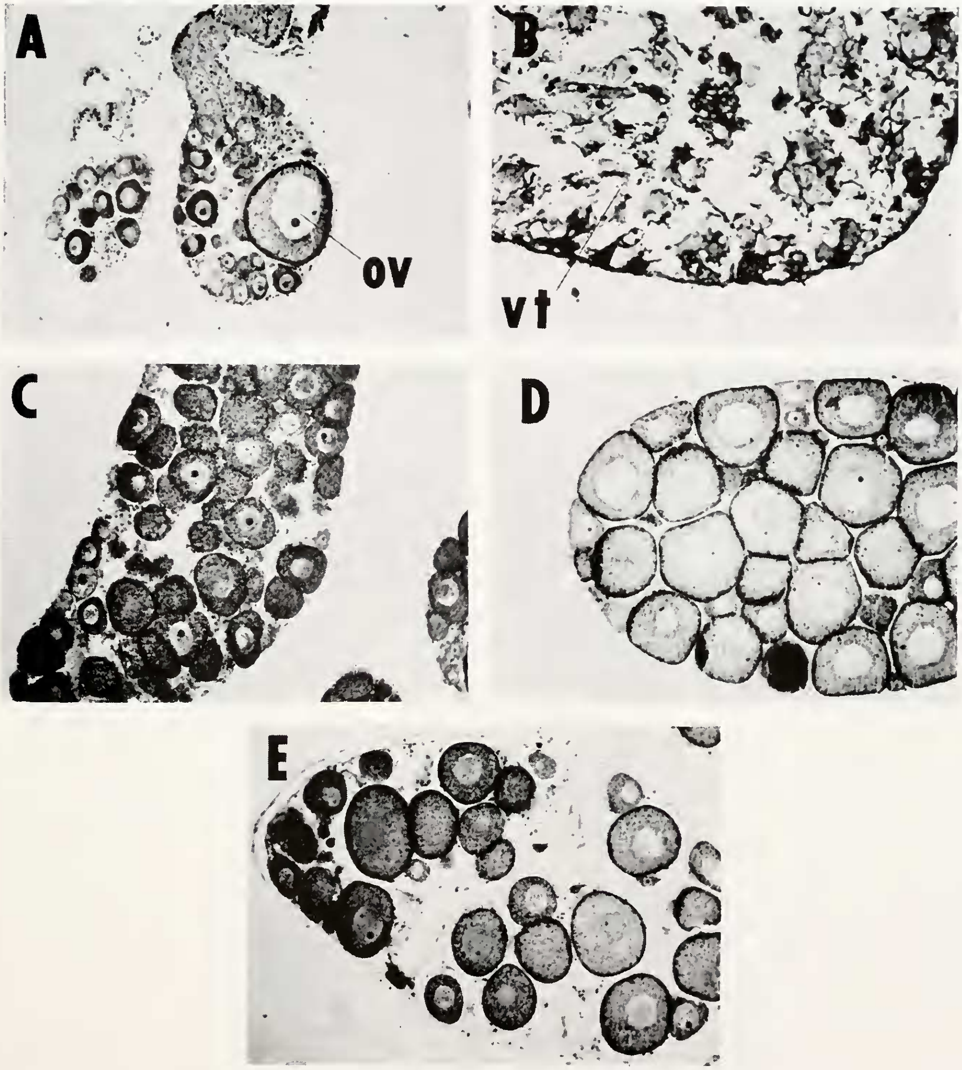

FIGURE 2. Histological sections of gonads of *Amphiura chiajei;* A. Stage 1 ovary, specimen collected in December 1965, ov = large oocyte remaining from the preceding maturation, × 300; B. Stage 1 ovary, October 1965, vt = vesicular tissue, × 300; C. Stage 2 ovary March 1966, × 120; D. Stage 3 ovary, June 1965, × 120; E. Stage 5 ovary, August 1965, × 120.

Summer 1965. There was no collection in the month of July. In August there are no more ophiuroids at stage 3, stages 4 and 5 are the only ones represented. Stage 5 is slightly more abundant than in June. In September, the majority of the ophiuroids are at the stage 4, some are at stage 1 and stage 5 is better represented than in August.

Autumn 1965. In October, stage 5 is the most abundant, stage 4 is still well represented and a few ophiuroids are at stage 1. In November and December the ophiuroids have gonads at stage 5, 1 and 2. Stage 5 which reaches a maximum in November, begins to regress in December and disappears completely by the end of February. Stages 1 and 2 become more abundant during autumn.

Winter 1966. The evolution observed since November is increased. Stage 1 reaches a maximum in February, then regresses in March. From February onwards, stage 3 is represented. It is predominant in March during which month some ophiuroids already reach maturity.

B. In females

Spring 1965. In April and May, stages 1, 2 and 3 are represented, but from May onwards a regression of stages 1 and 2 can be observed while the percentage of ophiuroids at stage 3 increases and reaches a maximum in June. As in males, some ophiuroids are at stage 5 at the end of spring (Fig. 3 B).

Summer 1965. During this season, stage 5 becomes most important. Some ophiuroids with gonads at stage 1 have been collected in August.

Autumn 1965. In October stage 3 is still well represented but it disappears in November. Stage 5, less abundant than in September, is still present until November. In December only stages 1 and 2 were observed.

Winter 1966. In January and February stage 1 is more abundant than stage 2. In March the two stages are in equal proportions.

The increase in size of the oocytes was followed by measuring in sections, the diameters of 50 oocytes according to the technique of Pearse (1965). The oocytes were classified in groups of ten microns (Fig. 4).

From April until June 1965, the average size of the oocytes increases progressively. In August it is slightly inferior to that of the month of June. This decrease in size is due to the fact that 35% of the ophiuroids are at stage 5 which is characterized by a loss of large oocytes and by a growth of young oocytes. In September, two modes are clearly distinguishable. The first mode is due mainly to females at stage 3, the second mode to females at stage 5, the stage during which there is growth of young oocytes. This situation is even more evident in October. Finally, in November, there is essentially only one mode in the frequency polygon: 80% of the females are at stage 1. The new generation of oocytes begins a period of growth which is almost linear until the month of March (Fig. 4).

In the males the appearance of stage 4 in the month of March indicates the beginning of the period of maturity and is followed shortly afterwards by the shedding period which begins in June, reaches a maximum in November and comes to an end at the beginning of winter. In the females the period of maturity probably begins in spring. As for males, stage 5 is present as early as June. As I already said, even if, in the gonads at stage 5 secondary oocytes are not seen, there is an evolution in comparison with the pre-maturation stage. This evolution is indicated by the appearance of vesicular phagocytotic tissue, the growth of a new generation of primary oocytes and the presence of large oocytes which have a degenerating cytoplasm. These characteristics suggest that the oocytes are expelled in a state of maturation. I shall discuss this possibility later. On the other hand, at the end of May and the beginning of June 1963, I observed egg-shedding in the laboratory. The embryos developed normally. It seems that the egg-shedding period begins in spring and finishes in November and thus, it is less extended than the period of sperm shedding.

Seasonal Cycle of Planktonic Larvae

Few planktonic larvae of *A. chiajei* were collected: 116 in 710 hauls made at a depth of 5 meters at two stations of Villefranche Bay. The first station was at the entrance of the bay over rocky beds more than 50 meters deep and the second in the interior of the bay above a bed of a marine Phanerogam, *Posidonia oceanica,*

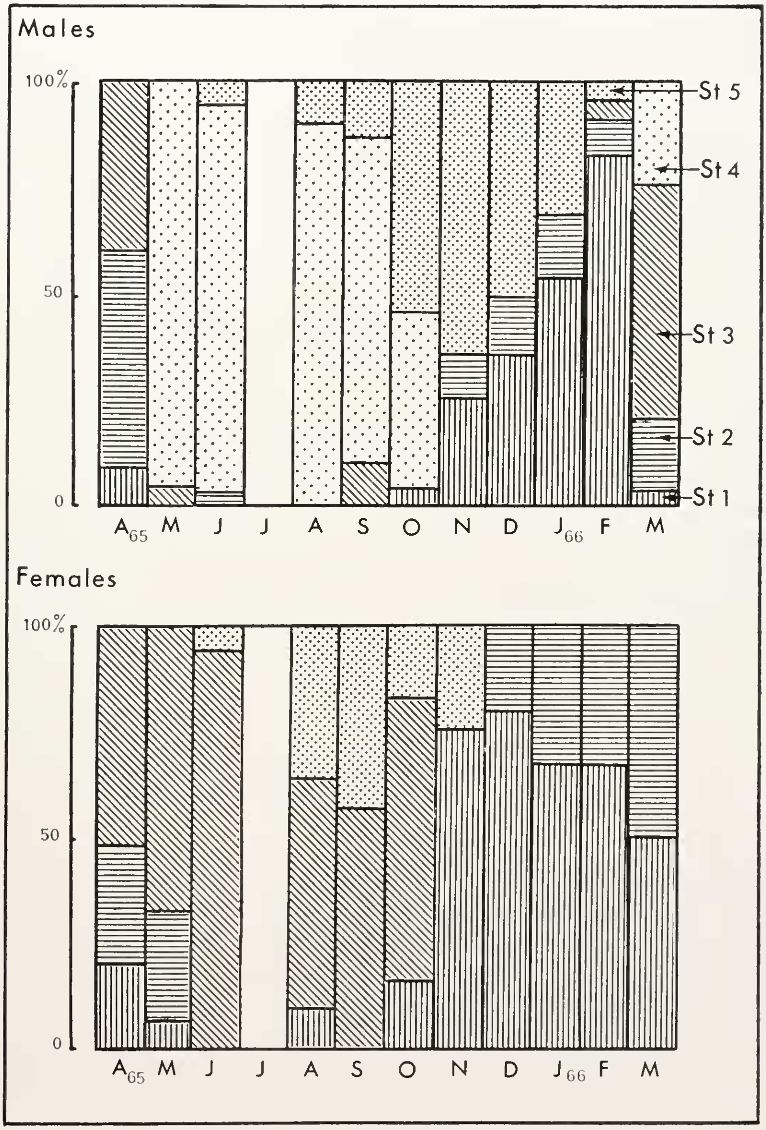

Figure 3. Relative proportion of animals in each gonad stage of *Amphiura chiajei.*

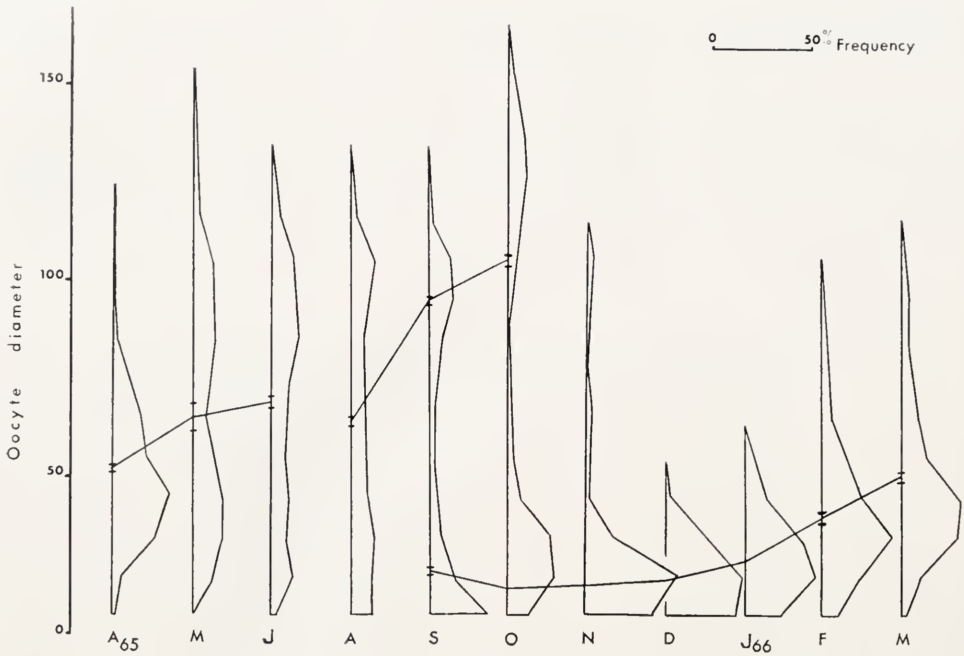

FIGURE 4. Evolution of the average size of oocytes (April 1965–March 1966).

25–30 meters deep (Fenaux, 1968 a). The annual temperature variation at this depth during the period studied was from 12° C to 16°C. Figure 5 represents the monthly averages for these four years. Autumn is the main period of the appearance of ophioplutei (Fig. 5). At station 1, one to two ophioplutei are found for ten planktonic hauls. At station 2, the number of ophioplutei harvested is greater because of the transport of larvae by currents from the abundant population of *Amphiura* in the interior basin of the bay. In August and especially in October and November quite large numbers of larvae are occasionally observed, more than ten per hauls, whereas the average catch is, as for station 1, one to three larvae for ten hauls. Several ophioplutei have been collected in winter and spring.

Nearly all the larvae collected were in the process of metamorphosis.

DISCUSSION

A histological examination of the gonads of *A. chiajei* has permitted me to state precisely the succession of the various stages of gametogenesis. After a period of sexual rest, the sex cells begin their development in winter (differentiation of the spermatids, growth of the primary oocytes) and come to maturity in spring. Reproduction takes place principally at the end of summer.

Concerning spermatogenesis, there is a relatively important phase of accumulation of spermatozoa. If the differentiation of spermatogonia into spermatids takes place during an extended period, such activity is only observed in a small percentage of the population.

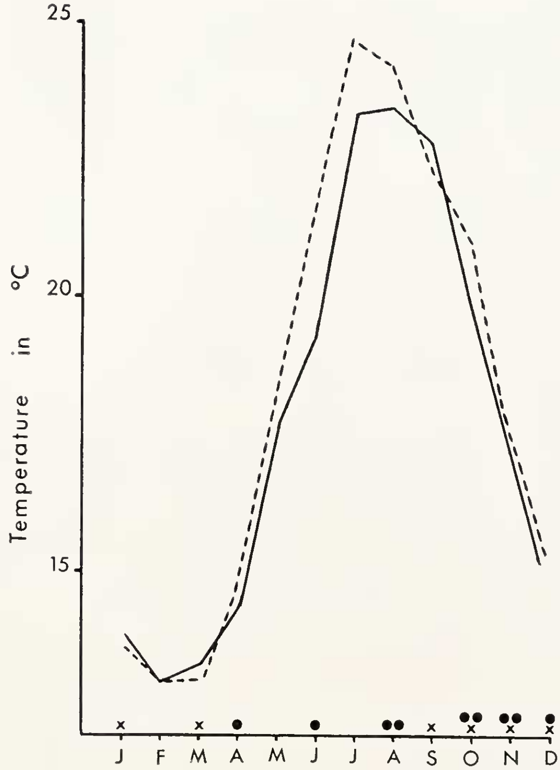

FIGURE 5. Presence of the *Amphiura chiajei* larvae in plankton hauls made at Station 1 (X) and Station 2 (●); X or ● = 1 to 2 ophioplutei for 10 planktonic hauls; ●● = 1 to 3 ophioplutei for 10 planktonic hauls; Continuous line curve: average temperature at 5 meters depth at Station 1 (January 1961–April 1964); Dotted line curve: average temperature at 5 meters depth at Station 2 (April 1961–April 1964).

As I pointed out above, although I have been able to observe the different stages of spermatogenesis, this is not true for oogenesis. In fact, no stage of maturation was disclosed in the numerous histological sections or smears which were examined. The shedding stages begin suddenly after a long period of primary oocytes growth. Moreover, the attempts made for artificial fertilization in June and in September, were unsuccessful. There was no expulsion of polar globules after the addition of spermatozoa, as was observed in the asteroid *Leptasterias hexactis* (Chia, 1968) or when the oocytes were liberated in water, as in the case of *Marthasterias glacialis.* It is probable that maturation is accomplished very rapidly within the gonad, and is completed only a short time before the ejection of the eggs. This would explain why the secondary oocytes are absent in the histological sections which were examined. Therefore, there is not, as in the males, a more or less protracted period of sexual maturation. This characteristic seems to be frequent in Ophiuroides; it was observed also, after a histological examination, in a Euryalae *Gorgonocephalus caryi* (Patent, 1969). Those in which artificial fertilization has been successful and which store their eggs for some time, are rare. Such rarity of secondary oocytes has been observed in other Echinoderms: *Odontaster validus* (Pearse, 1965), *Stylocidaris affinis* (Holland, 1967). According to Holland (1967), in Euechinoids and the Echinoderms apart from the Echinoids, after a very lengthy growth period of the primary oocytes, a rapid maturation occurs when the maximum size is reached. My observations on *A. chiajei* confirm this point of view.

Further information regarding the egg-shedding period is provided by the examination of plankton hauls. This period extends mainly from the end of summer until autumn; however, a few ophioplutei have been caught at the end of winter and in spring. Few planktonic larvae were collected. In the laboratory, I obtained a complete larval development, and the formation of a young ophiuroid whose larval appendages were completely reduced (Fenaux, 1963) within eight days at a temperature of 18°–20° C. Such brevity of larval life is probably the main cause of the poverty of ophioplutei in the plankton hauls. For the eggs laid at the end of summer and at the beginning of autumn in water in which the temperature is superior to 20° C, the pelagic life is probably even shorter.

I have described the larvae which develop from fertilized eggs obtained from a natural egg-shedding (Fenaux, 1963). They are identical to planktonic larvae: the differences in size between the planktonic ophioplutei and those reared in the laboratory are minimal. Only the post-lateral appendages are longer in planktonic larvae.

	Planktonic larvae	Larvae reared in lab.
Average length of body	260 microns	276 microns
Average length of post-lat. rods	472 microns	393 microns
Average length of somatic rods	88 microns	87 microns

It is possible to relate *A. chiajei* to a certain number of larvae previously described; for example the Bohuslän planktonic larva described by Mortensen (1920), who related it to *Ophiura affinis*. According to this author, color is the only notable difference between the ophiopluteus found at Bohuslän and those of the Adriatic described by Müller in 1853. Müller connected this larva with *A. chiajei* (which he called *Ophioplepis sundevalli*). I have compared Müller's drawings and the *A. chiajei* larva that I have reared, and, most probably, it is the same ophiopluteus. Chadwick (1914, page 184, Figures 46 and 46A of the Pl. III) reported larvae found in the plankton hauls carried out at Port Erin in the month of December. All the samples of this larva were in the process of metamorphosis. It seems that this larva also should be connected with *A. chiajei*. It shows numerous common characteristics with the ophiopluteus of this species, especially in junction of the somatic and the postero-lateral rods.

Taking these findings into account we can state precisely the periods of reproduction of *A. chiajei* in different regions. In Sweden, the egg-laying is in the middle of September (Mortensen, 1920) but it can be earlier, the larva described by Mortensen having been caught in August. In Port Erin, egg-laying probably takes place at the end of autumn. In the Mediterranean, at Naples, the period of maturity extends from May until September (Lo Bianco, 1909), at Villefranche in spring and in summer, with egg-shedding beginning at the end of summer.

Finally, in the Adriatic, the reproduction takes place in October (Vatova, 1950). This appearance of larvae at the end of summer in the Mediterranean Sea and in the Boreal areas shows that a determined temperature has not induced the maturation of eggs.

Summary

1. The seasonal evolution of the gonads of *A. chiajei* during the period from April 1965 until March 1966 has been described. The reproductive cycle allows a period of sexual rest at the end of autumn, a period of growth in winter, a period of maturity in spring and in summer, and an egg-shedding period from the end of summer until the middle of autumn.

2. The seasonal cycle of planktonic larva was studied during a period of three years. The larvae appear in autumn; however, a few of them were caught in spring and in summer.

3. The problem of maturation of the primary oocytes is discussed. The larvae described by Müller (1853), Mortensen (1920) and Chadwick (1914) are related to *A. chiajei*. The periods of reproduction of this species in various Northern and Mediterranean regions are specified.

LITERATURE CITED

Chadwick, H. C., 1914. Echinoderm larvae of Port Erin. *Proc. Trans. Liverpool, Biol. Soc.*, **22**: 467–498.

Chia, F. S., 1968. The embryology of a brooding starfish Leptasterias hexactis. *Acta Zoologica*, **XLIX**: 321–364.

Delavault, R., 1960. La sexualité chez Echinaster sepositus Gray du Golfe de Naples. *Pubbl. Sta. Zool. Napoli*, **32**: 41–45.

Fenaux, L., 1963. Note préliminaire sur le développement larvaire de Amphiura chiajei Forbes. *Vie Milieu*, **XIV** (1): 91–96.

Fenaux, L., 1968 a. Maturation des gonades et cycles saisonniers des larves chez Arbacia lixula, Paracentrotus lividus et Psammechinus microtuberculatus (Echinides). *Vie Milieu*, **XIX** (1 A): 1–52.

Fenaux, L., 19628 b. Aspects écologiques de la reproduction des Echinides et Ophiurides de Villefranche-sur-Mer. Thèse d'état.

Fuji, A., 1960. Studies on the biology of the sea urchin. I. Superficial and histological changes in gametogenic process of two sea urchins, Strongylocentrotus nudus and S. intermedius. *Bull. Fac. Fish. Hokkaido Univ.*, **11**, (1): 1–14.

Gabe, M., and Martoja-Pierson, M., 1957. Sur une coloration trichrome en un temps sans différenciation. *Bull. Microsc. Appl.* **7**: 60–63.

Holland, N. D., 1967. Gametogenesis during the annual reproductive cycle in a cidaroid sea urchin (Stylocidaris affinis). *Biol. Bull.*, **133** (3): 578–590.

Lo Bianco, S., 1909. Notizie biologiche riguardante specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli. *Mitt. Zool. Sta. Neapel*, **19** (4): 556–566.

Mortensen, Th., 1920. Notes on the development and larval forms of some Scandinavian Echinoderms. Vidensk. Medd. Dansk. *Naturhist. Foren. Kjobenhaun*, **71**: 133–160.

Müller, J., 1852 (1853). Über den allgemeinen Plan in der Entwickelung der Echinodermen. *Abh. Deut. Akad. Wiss. Berlin*, **1853**: 123–219.

Patent, D. H., 1969. The reproductive cycle of Gorgonocephalus caryi (Echinodermata; Ophiuroidea). *Biol. Bull.*, **136**: 241–252.

Pearse, J. S., 1965. Reproductive periodicities in several contrasting populations of Odontaster validus Koehler, a common antarctic asteroid. Biology of the Antarctic Seas. II. *Antarctic Res. Series* **5**: 39–85.

Vatova, A., 1950. Gli Echinodermi delli Laguna Venta. *Nova Thalassia*, **I** (7): 3–13.